CASE REPORT

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Multiple myeloma with high adenosine deaminase expression

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A 50-year-old man with immunoglobulin A type multiple myeloma (MM) was referred to our hospital after bortezomib therapy. He had high alkaline phosphatase and lactate dehydrogenase levels. Computed tomography showed osteolytic and osteoblastic bone lesions. Response to salvage chemotherapy was temporary, and he developed a right pleural effusion with high adenosine deaminase (ADA) levels. He died from bleeding associated with a pelvic bone fracture 9 months later. ADA mRNA expression and ADA secretion of the MM cells from the patient were higher than those from myeloma cell lines tested. Clinical relevance of high ADA expression in MM cells is warranted.

Key words: adenosine deaminase, multiple myeloma, pleural effusion

Introduction

Multiple myeloma (MM) is characterized by the accumulation of neoplastic plasma cells in the bone marrow which generates multiple bone lesions through enhanced osteoclastogenesis and concomitant suppression of osteoblastic differentiation from the bone marrow stromal cells. MM is still difficult to be cured; therefore, additional novel treatment strategies are needed.

Adenosine deaminase (ADA) is an important enzyme in the purine metabolism that catalyzes the deamination of both adenosine and 2′-deoxyadenosine to inosine and 2′-deoxyinosine, respectively [1]. However, limited information is available regarding ADA production and its role in MM.

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Here, we report a patient with MM exhibiting an aggressive clinical course with a pleural infiltration of MM cells expressing high levels of ADA.

Case Report

A 50-year-old man presented with general fatigue, appetite loss, and pain in shoulders, back, and ribs from December 20XY. He was diagnosed with MM in March next year. He had hypercalcemia, renal insufficiency, and serum immunoglobulin A (IgA)-λ type M-protein at 2420 mg/dl with multiple osteolytic and osteoblastic lesions. In addition, 88% of bone marrow cells were occupied by MM cells. After 2 courses of bortezomib, dexamethasone, and cyclophosphamide combination therapy, he was referred to our hospital for subsequent treatment. Urinalysis revealed proteinuria, whilst peripheral blood tests showed normocytic anemia, high alkaline phosphatase (ALP), high lactate dehydrogenase (LDH) and IgA level of 608 mg/dl. Bone metabolic markers revealed high levels of bone resorption and formation activity (Table 1). A chest X-ray scan showed multiple extramedullary tumors in both lungs. Computed tomography revealed systemic bone lesions. Most notably, osteolytic and osteoblastic lesions were intermingled in spinal bones and the sternum (Fig. 1a, 1b, and 1c). Bone marrow MM cells were immature in appearance with a CD38 (+), CD138 (+), CD19 (–), CD56 (+), CD20 (–), CD33 (–), CD49e (–), and MPC-1 (–) phenotypes (Fig. 1d). Fluorescent in situ hybridization analysis revealed complicated karyotypes with deletion of chromosomes 1,13q and 17 together with (4;14) translocation in the bone marrow cells.

Table 1. Laboratory findings on admission

<Peripheral blood>			<Blood chemistry>			<Immune serology>			<Bone metabolism markers>		
WBC	4000	/μl	T-Bil	0.4	mg/dl	IgG	278	mg/dl	BAP	173.0	μg/l
stab	1.0	%	AST	34	U/l	IgA	608	mg/dl	Osteocalcin	32.0	ng/ml
seg	75.0	%	ALT	14	U/l	IgM	27	mg/dl	TRACP-5b	1180	mU/dl
baso	1.0	%	ALP	1831	U/l	CRP	<0.05	mg/dl	uDPD	602.0	nmol/mmolCr
eosino	2.0	%	γ-GTP	26	U/l	β_2MG	3.86	mg/l	1CTP	41.6	ng/ml
mono	5.0	%	LDH	706	U/l	FLC-κ	12.8	mg/l	NTX	84.5	nmolBCE/l
lymph	16.0	%	TP	7.5	g/dl	FLC-λ	315	mg/l	uNTX	480.0	nmolBCE/mmolCr
other	0.0	%	Alb	4.1	g/dl	κ/λ	0.04				
RBC	341×10^4	/μl	BUN	9	mg/dl						
Hb	10.9	g/dl	Crn	0.75	mg/dl						
Ht	32.9	%	UA	5.9	mg/dl						
MCV	96.4	fL	Na	142	mEq/l						
MCH	32.3	pg	K	3.5	mEq/l						
PLT	22.2×10^4	/μl	Cl	101	mEq/l						
			Ca	9.1	mg/dl						
			IP	3.3	mg/dl						
			PG	95	mg/dl						

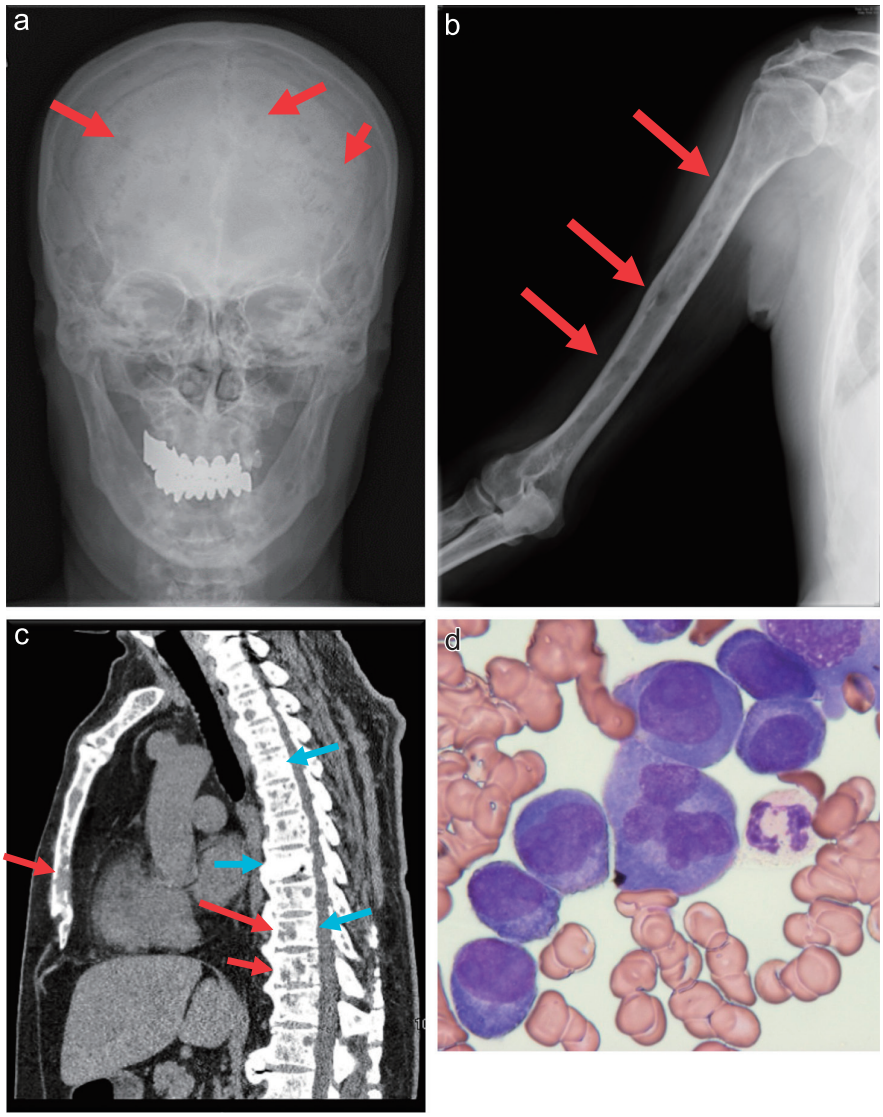

Figure 1. Myeloma bone lesions and morphology of myeloma cells. a: Multiple punched out lesions are observed in an X-ray scan of the skull (red arrow). b: Osteolytic myeloma bone disease in the right upper arm (red arrow). c: Osteogenic myeloma bone disease in the vertebral body (blue arrow), osteolytic bone disease in the vertebrae and sternum (red arrow). d: Myeloma cells observed in the bone marrow.

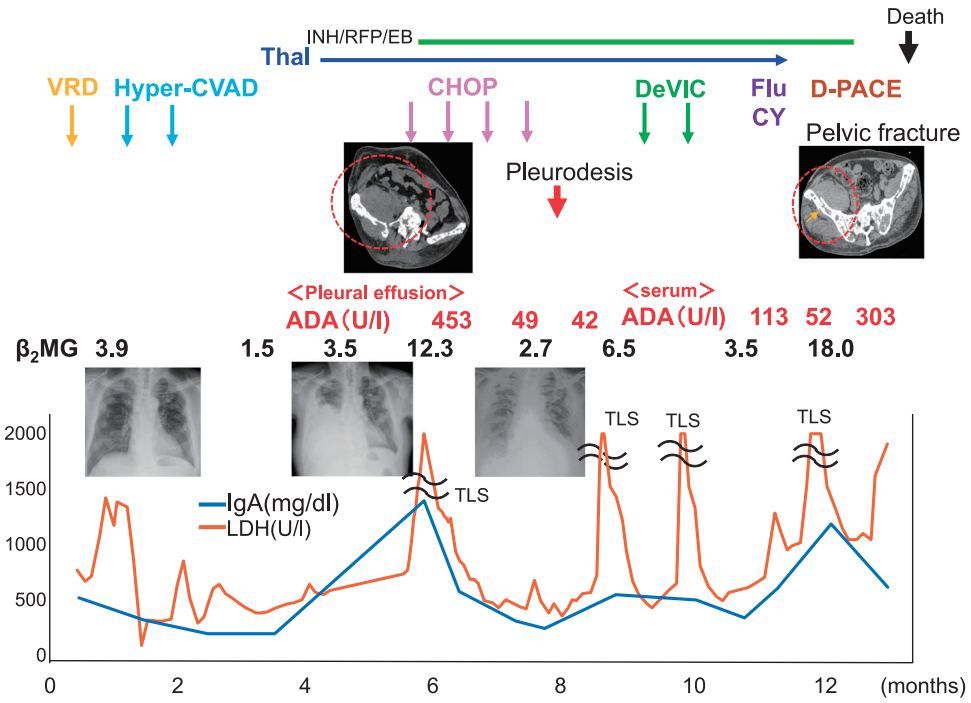

Figure 2. Clinical course. After hyper-CVAD therapy as a salvage therapy, a right pleural effusion with high ADA expressive myeloma cells developed. Patient underwent talc pleurodesis to palliate his respiratory symptoms. CHOP, DeVIC and D-PACE salvage chemotherapy induced repetitive TLS, however, anti-tumor effect was temporary. The patient died from a pelvic bone fracture after 13 months after diagnosis of myeloma.

Abbreviations: ADA = adenosine deaminase; CHOP = cyclophosphamide, doxorubicin, vincristine, and prednisone; DeVIC = dexamethasone, etoposide, ifosfamide and carboplatin; D-PACE = dexamethasone, cisplatin, doxorubicin, cyclophosphamide and etoposide; hyper-CVAD = hyperfractionated cyclophosphamide, vincristine, doxorubicin (Adriamycin), and dexamethasone; IgA = immunoglobulin A; LDH = lactate dehydrogenase; TLS = tumor lysis syndrome.

Bortezomib, lenalidomide, and dexamethasone combination chemotherapy was not effective; however, two salvage courses of hyperfractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone (hyper-CVAD) therapy followed by thalidomide monotherapy appeared effective; and the patient achieved a partial response. ALP was lowered to normal range after hyper-CVAD therapy; however, LDH and IgA levels resumed to increase immediately followed by rapid expansion of a right pleural effusion. Examination of the pleural effusion revealed a high level of ADA (453 U/L) with abundant myeloma cells. *Mycobacterium* species were not detected in the pleural effusion. Computed tomography scans showed multiple extramedullary expansion of tumors with a bulky mass. Combination chemotherapy of cyclophosphamide, doxorubicin, vincristine, and prednisone was temporarily effective as subsequent salvage chemotherapy although a tumor lysis syndrome emerged. He underwent a talc pleurodesis to palliate his respiratory distress. Salvage chemotherapy regimens of dexamethasone, etoposide, ifosfamide and carboplatin; fludarabine; dexamethasone, cisplatin, doxorubicin, cyclophosphamide and etoposide were only temporarily effective. The patient died from bleeding, resulting from a pelvic bone fracture (Fig. 2), only 13 months after the diagnosis and 9 months after the emergence of the pleural effusion. Serum levels of LDH and ADA were found to reflect a MM tumor expansion.

Discussion

Herein, we described an MM patient with poor risk chromosomal abnormality and severe bone lesions who quickly developed a right pleural effusion with high ADA mRNA expression and secretion from MM cells (Fig. 3). The progressive nature of his condition and his resistance to chemotherapy eventually lead to his death.

Pleural effusion in myeloma is not uncommon; Kintzer et al. reported that 6% of MM cases develop pleural effusion [2]. Most cases of effusion are derived from renal failure, heart failure, hypoalbuminemia, and infection. However, malignant pleural effusion only occurs in 1% or less of MM cases, making it relatively rare [2]. In our case, the patient developed unilateral pleural effusion with MM cell infiltration and high ADA in the supernatant of the effusion. Repeated examination ruled out tuberculosis, and we verified in vitro that ADA was pro-

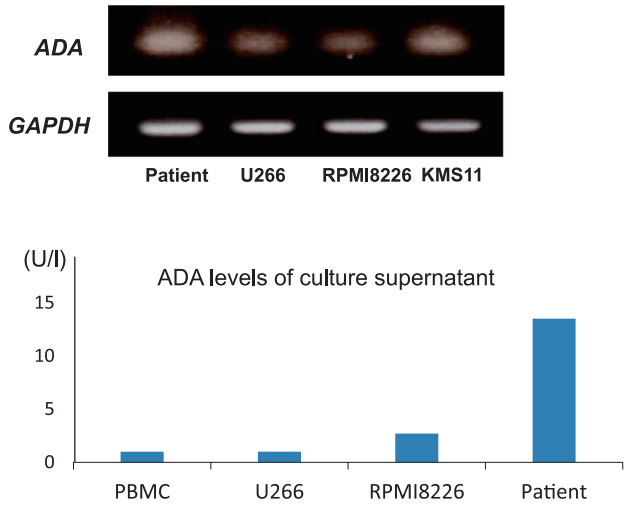

Figure 3. Expression of ADA in MM cells. MM cells from his pleural effusion were purified with positive selection using CD138 (Syndecan-1) microbeads and the Miltenyi magnetic cell-sorting system (Miltenyi Biotec, Auburn, CA), according to the manufacturer's instructions. The MM cells were then cultured, and total RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA). For reverse transcription–polymerase chain reaction as previously described. The specific primers used were forward 5′-TTCCTTCCAAGAAGACCATGA-3′, reverse 5′-GGTTTCAGATTCA ACCATGC-3′, for human ADA, forward 5′-TGTCTTCACCACCATG GAGAAGG-3′ and reverse 5′-GTGGATGCAGGGATGATGTTCTG-3′ for human glyceraldehyde-3-phosphate dehydrogenase. MM cells were cultured for two days at 1×10^6 cells/ml in RPMI 1640 medium with 5% fetal bovine serum. The supernatants were collected and their ADA levels were measured. All procedures involving human specimens were performed with written informed consent according to the Declaration of Helsinki, and the protocol was approved by the Institutional Review Board for human protection in Tokushima University hospital (approval No. 240-2). ADA mRNA expression and production of the patient's MM cells from the pleural effusion were significantly higher compared to those in peripheral blood mononuclear cells (PBMCs) from a healthy donor and MM cell lines tested.
Abbreviations: ADA = adenosine deaminase; PBMCs = peripheral blood mononuclear cells.

duced by MM cells in the pleural effusion because both transcriptional and protein levels were much higher than in other MM cell lines (Fig. 3). In a clinical setting, ADA is used as a diagnostic marker for tuberculous pleuritis, peritonitis, and meningitis because ADA protein expression in these effusions is known to be positively correlated with tuberculosis [3–5]. The role of ADA in MM is unclear, and to date, only 7 patients with MM and high ADA expression have been reported [6–8]. All of them developed pleural effusion during their treatment. Six of seven patients were under sixty-year-old, markedly young. The IgD isotype was seen in 3 cases, and the IgG and IgA isotypes were in 2 cases respectively. Bone disease and monosomy 13 or del(13q) were seen in three cases (Table 2). In our case, the ADA level from the effusion was the highest among all previously reported cases. Period of their survival was reported to be between only a month and 1 year.

ADA is a critical enzyme expressed in all tissues of human body that catalyzes the deamination of both adenosine and 2′-deoxyadenosine to inosine and 2′-deoxyinosine, respectively [1]. ADA is also regarded as an activation marker of lymphoid cells, and the absence of ADA results in both intra- and extracellular accumulation of adenosine, which leads to lymphocyte apoptosis and immune deficiency [9]. High ADA expression reflects high purine metabolism activity; therefore, ADA is thought to be a probable target for the inhibiting cancer metabolism. A number of ADA inhibitors have been synthesized [10], and some are approved for their clinical use, including cladribine for hairy cell leukemia and relapsed/refractory follicular or mantle cell lymphoma as well as pentostatin for hairy cell leukemia and adult T-cell leukemia. However, ADA inhibitors has not yet been studied in MM.

Adenosine, a substrate of ADA, has drawn considerable attention for its association with cancer. Adenosine activates the adenosine receptors on the target cell, which generate various cellular responses, including immune cell regulation and tumor cell proliferation that result in alteration of the

Table 2. Reported myeloma cases with high ADA expression in the pleural effusion

Ref.	Age	Gender	Type	ADA (U/l)	Bone disease	Monosomy13 or del (13q)	Survival after pleural effusion appearance (month)
6	49	M	IgA-κ	61	ND	ND	11
7	58	M	IgD	50	ND	ND	1
8	53	M	IgD-λ	68.4	osteolytic	+	3.7
8	53	F	IgA-κ	84.2	—	ND	0.7
8	53	M	IgG-κ	108.5	osteolytic	+	0.8
8	67	M	IgD-λ	117.8	osteolytic	+	15.6
8	48	F	IgG-κ	60.6	—	–	4.1
present case	55	M	IgA-λ	453.0	osteolytic and osteoblastic	+	9

ND: not described

tumor microenvironment [11]. On the other hand, deaminase activity also seems to be important in cancer. The adenosine deaminase acting on RNA (ADAR) family genes and proteins deaminate adenosine to inosine in double-stranded RNA. They were discovered through the purification of *Xenopus*, bovine liver, and calf thymus [12–14] . This reaction is commonly referred to as Adenosine-to-Inosine RNA editing, a highly conserved mechanism in mammals [15]. ADAR is reported to affect various human biochemical activities, such as mRNA translation, RNA stability, RNA replication, and RNA silencing, which involves hematopoiesis and pluripotency of hematopoietic stem cells [16]. Although it is unclear whether deamination activity influences on MM progression, we need further studies on adenosine or its deamination-related pathophysiology and tumor pathophysiology in MM.

Bone disease in our case showed a characteristic feature with simultaneous osteoblastic and osteoclastic bone lesions when he was referred to our hospital. In humans, osteoblast precursor produce adenosine that has a potent stimulatory action on IL-6 secretion but an inhibitory action on osteoprotegerin [17]. In addition, adenosine signaling is reported to stimulate human osteoblastogenesis [18] and osteoclastogenesis [19]. Therefore, adenosine or ADA activity might affect the characteristic phenotype of bone disease in our patient.

In summary, we described a patient with MM showing high ADA expression with bone disease mixed with osteolytic and osteoblastic lesions and unilateral pleural effusion. Because the role of ADA in MM pathophysiology and MM bone lesions remains unknown, further clinical experiences and laboratory investigations are warranted.

Conflicts of Interest Disclosures

The authors declare no competing financial interests related to this work.

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